

PURDUE UNIVERSITY  
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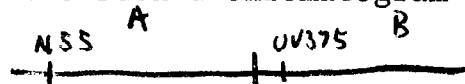
Oct. 9, 1966

Dear Francis,

Although I had vowed never again to look for rII, I broke down last winter when a bright and energetic young student by the name of William McClain started to work for me and could not be dissuaded from having a look. We decided it might be easier to detect a tryptic peptide of the rII protein rather than the protein itself if a labeled amino acid were used which occurs rarely in proteins but which pretty surely is in rII. Tryptophan was a good bet because there are rII ambers which are weakly or not at all responsive to 5FU and these should have arisen from tryptophan.

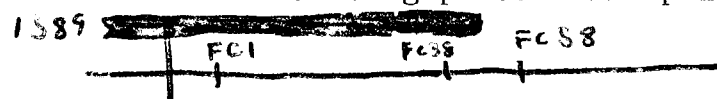
The initial approach was to chromatograph double labeled tryptic digests of the entire infected cell, comparing a nonsense mutant far to the left in A ( $H^3$ ) with a nonsense mutant far to the left in B ( $C^{14}$ ). A trypt requiring, tryptophanaseless, non-permissive host was used, and at the beginning we used only ochres since this host had an amber suppressor. The cells from the two cultures were centrifuged after labeling, mixed, and taken up in cold 90% formic acid. After dialysis, the material was boiled, digested with trypsin and chromatographed on Technicon peptide resin (essentially Dowex 50).

The attached Fig. 1 shows such a chromatogram comparing N55 with UV375.



The  $H^3$  and  $C^{14}$  track very closely except in the region around fraction 125 (component 30) where there is a striking increase in the  $H^3$  to  $C^{14}$  ratio, suggesting that component 30 is a product of the B cistron. Note that the control N55 ( $H^3$ ) vs N55 ( $C^{14}$ ) shows no such peak.

Our attempts to locate the region controlling component 30 are summarized in Fig. 2 where + means that the mutant makes 30 and 0 means that it doesn't. Note that 1589 fails to produce 30 indicating that 1589 overlaps the controlling region. This is consistent with further results using phase shift pairs FC(1,38) and FC(1,58)



shown in Fig. 3. The fact that the former pair is + while the latter pair is 0 implies that the left terminus of the region is between FC38 and the end of 1589.

(For this experiment an A cistron mutation was inserted into the FC doubles to prevent phage production and possible secondary consequences.)

Fig. 4 is a plot of two separate single label runs showing that double label is not really necessary and that the effect is not some funny business due to double label counting. That component 30 is in fact a peptide has been shown by recovering tryptophan fairly quantitatively after pronase digestion.

We have tried to produce a chromatographically altered component 30 by looking at presumed missense mutations within the controlling region but have not been successful- either it is present or absent. Difficulties arise in re-chromatography of comp 30 for further analysis since it is often altered by the manipulations (probably drying) required to concentrate enough of it for this purpose.

Our main aim now is to use component 30 as an assay for the intact protein and thereby try to purify the intact protein. This is just beginning and there is nothing yet to report. I think this approach offers the best hope of obtaining the left tip of the B protein.

I would certainly be most interested in having a copy of your manuscript. The authoritative map would be very useful to have. I have a preliminary one from Drake who said there were probably some inaccuracies. Our assumption that FC 58 is to the right of 1589 came from this map and I wonder if this is really true. Our crosses of 1589 with FC 58 have not convinced me that they are separate. In any case it would be useful to have an additional +- pair like (1,58) but where the right mutant is further out than 58 if you could supply one. Also if you will send X655 we will see if it makes component 30.

Best regards,

A handwritten signature in cursive script, appearing to read "Sewell".